

Development of a Host-Based Semiochemical Lure for Trapping Emerald Ash Borer *Agrilus planipennis* (Coleoptera: Buprestidae)

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ABSTRACT Bark volatiles from green ash *Fraxinus pennsylvanica* were tested for electrophysiological activity by *Agrilus planipennis* using gas chromatographic-electroantennographic detection (GC-EAD) and for behavioral activity using baited purple traps in Michigan. GC-EAD analysis of the headspace volatiles of bark tissue samples from 0- and 24-h-old fully girdled (stressed) ash trees showed that the latter had elevated sesquiterpene levels. Six of the elevated compounds consistently elicited antennal responses by both male and female *A. planipennis*. Five of the antennally active compounds were identified as α -cubebene, α -copaene, 7-*epi*-sesquithujene, *trans*- β -caryophyllene, and α -humulene (α -caryophyllene). The sixth EAD-active compound remains unidentified. We monitored capture of adult *A. planipennis* on traps baited with several combinations of ash tree volatiles. Treatments included two natural oil distillates (Manuka and Phoebe oil) that were found to contain, respectively, high concentrations of four and five of the six antennally active ash bark volatiles. A four-component leaf lure developed by the USDA Forest Service and Canadian Forest Service was also tested. In three separate field studies, Manuka oil-baited traps caught significantly more adult beetles than unbaited traps. Lures designed to release 5, 50, and 500 mg of Manuka oil per day all caught more insects than unbaited traps. In a field test comparing and combining Phoebe oil with Manuka oil, Phoebe oil-baited traps caught significantly more beetles than either Manuka oil-baited traps or unbaited traps. We hypothesize that the improved attractancy of Phoebe oil to *A. planipennis* over Manuka oil is caused by the presence of the antennally active sesquiterpene, 7-*epi*-sesquithujene.

KEY WORDS Buprestidae, bark volatiles, 7-*epi*-sesquithujene, Manuka oil, Phoebe oil

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive pest of ash (*Fraxinus* spp., L.) (Oleaceae) that was first discovered in and around Detroit, MI, and Windsor, Ontario, Canada (Haack et al. 2002).

Within 2 yr of the insect's discovery, $\approx 650,000$ landscape ash trees within a 6,475-km² area were found to be infested in the United States (Rauscher and Mastro 2004), as well as 100,000 trees in Canada (Marchant 2004). The area infested with *A. planipennis* now exceeds 40,000 mi² in Michigan, Ohio, Indiana, Illinois, Maryland, Pennsylvania, West Virginia, and Ontario, Canada (EAB 2007).

Although *A. planipennis* is considered a minor pest within its native range of eastern Asia (Yu 1992), in North America it is attacking and killing green ash [*F.*

pennsylvanica variety *subintegerrima* (Vahl) Fern.], white ash (*F. Americana* L.), and black ash (*F. nigra* Marsh.). It is estimated that 15 million ash trees in forests, woodlots, and urban settings are dead or dying as a result of *A. planipennis* in Michigan alone (Poland and McCullough 2006). Larval feeding produces serpentine galleries that disrupt nutrient flow in the phloem, usually resulting in tree death within 2–3 yr after initial attack (Liu et al. 2003). Movement of infested firewood and nursery trees has helped facilitate the spread of this pest (Marchant 2006). Continued spread of *A. planipennis* through North America threatens at least 16 endemic ash species (USDA NRCS 2004, Wei et al. 2004).

An attractant-baited survey trap capable of detecting *A. planipennis* adults at low densities would be a potentially useful tool for detecting new infestations and monitoring management areas. Early detection of *A. planipennis* infestations has depended on visual inspection of trees. However, two signs used to identify infested trees—crown dieback and bark splits—are only noticeable during later stages of infestation, and exit holes in the bark surface are initially high in the canopy and difficult to detect during the early stages (Cappaert et al. 2005). Early infestations there-

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fore tend to go unnoticed (Haack et al. 2002). Sensitive methods of detecting populations of *A. planipennis* are needed for effective management of this pest (Francese et al. 2005).

There is little information at this time on how buprestids locate potential mates and hosts. For buprestids that have been studied, it seems that mate location is facilitated by host selection followed by visual, tactile, and possibly auditory cues rather than using pheromones over any distance (Carlson and Knight 1969, Gwynne and Rentz 1983). Oliver et al. (2002) showed that colors in the violet to pink range (i.e., 400–450 nm) were attractive to buprestids. In the most recent study on developing a trap for *A. planipennis*, Francese et al. (2005) showed that purple panel traps caught significantly more beetles than black, green, dark blue, red, silver, white, or yellow traps.

Studies have shown that the two-lined chestnut borer *A. bilineatus* attacks stressed oaks but not healthy or dead trees (Dunbar and Stephens 1976, Cote and Allen 1980, Haack and Benjamin 1982, Dunn 1985). Adults can locate oaks within 24 h of inducing a stress injury (Dunn 1985), strongly suggesting that tree-stress volatiles play an important role in tree colonization. Similarly, girdled (stressed) ash trees were found to be more attractive to *A. planipennis* than the healthy trees or cut logs (Poland et al. 2004, 2005).

Rodriguez-Saona et al. (2006) found that stressed ash seedlings were more attractive than healthy ash seedlings to females in laboratory bioassays. They identified several foliar volatiles that were elevated in stressed ash seedlings and elicited antennal responses by *A. planipennis*. To date, no studies have identified or examined ash bark volatiles that may be important in host attraction by *A. planipennis*.

Our main objectives were to compare volatile profiles from bark samples of healthy and girdle-wounded green ash trees and identify potential attractants using coupled gas chromatography-mass spectrometry (GC-MS) and coupled gas chromatographic-electroantennographic detection (GC-EAD). We wanted to locate sources of these compounds and test beetle attraction in the field. Recent studies by Francese et al. (2007) have shown that purple prism traps caught significantly more adult *A. planipennis* when positioned 13 m high in the tree (mid-canopy) compared with traps hung at 1.5 m. We therefore wanted to examine our lure treatments at different heights.

Materials and Methods

Insects. Infested ash logs were collected in July 2004 from a site in Whitmore Lake, MI, where infested wood was processed and disposed of. The infested logs were then taken to the USDA laboratory in Brighton, MI, where they were stored at 5°C to suspend beetle development until adults were needed. Late-instar larvae and pupae were carefully extracted from the xylem tissue before being shipped to the USDA-APHIS-PPQ Pest Survey Detection and Exclusion Laboratory quarantine facility at Otis ANGB, MA. Pupae

were kept in a dark container at room temperature until they emerged 3–4 wk later. Adults were fed fresh green ash foliage [*F. uhdei* (Wenzig) Lingelsh] in plastic 16-oz drinking cups (Solo, Urbana, IL), with water in 1-oz plastic cups fitted with a cotton wick. Insects were fed for at least 10 d before they were used for electrophysiological experiments.

Collection of Bark Volatiles. Volatiles from bark of three green ash, *F. pennsylvanica*, were collected using methods similar to those of Zhang et al. (2000). After removing any moss or lichens, a 15-cm-wide strip of outer bark and phloem tissue was removed around the circumference of each tree at breast height (≈ 1.4 m above ground) on 28 July 2004. Removed bark tissue was enclosed in a plastic cooking bag (polyacetate; 40.6 by 44.5 cm; Reynolds, Richmond, VA) with an activated charcoal filter tube in the air inlet. The volatiles in the bag were collected on Super Q (50/80 mesh; 300 mg in a glass tube; ID 3 by 110 mm) for 14 h (airflow 300 ml/min) using battery-operated pumps (Sensidyne, Clearwater, FL) and recovered in 1 ml of hexane (Omni-Solv). All aeration and solvent extracts were kept at -20°C before GC-MS or GC-EAD analyses. Because collection of initial bark samples resulted in fully girdling the trees and stressing them, we wanted to see if stressed bark aerations contained any new compounds 24 h later. We sampled bark tissue again from the same trees 1 d later (29 July 2004) from ≈ 10 cm above the initial girdle wound.

GC-MS Analysis. Initial chemical analyses were conducted using a combined Agilent Technologies 6890 network gas chromatograph (NGC) and 5973 mass-selective detector (GC-MSD). The GC was equipped with a J & W DB-1 column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm ; run in splitless mode). Helium was used as the carrier gas at a constant flow rate of 0.7 ml/min. The injector temperature was 250°C . Oven temperature was held at 45°C for 1 min, programmed to 280°C at $10^{\circ}\text{C}/\text{min}$, and held for 15 min. Volatiles were identified by comparison of the retention indices and mass spectra with those of available authentic synthetic compounds and a computerized data library (NIST version 2.0, 2002). The identification was verified by separation of volatiles (splitless injection) on a 30-m by 0.25-mm i.d. by 0.25- μm film HP-5MS column and analysis on Agilent Technologies 5973 mass-selective detector interfaced with a 6890 N GC. Helium was used as the carrier gas at 1 ml/min. Oven temperature was held at 120°C for 14 min, programmed to 280°C at $11^{\circ}\text{C}/\text{min}$, and held for 2 min. GC analyses were also performed on a HP-5 column (30-m by 0.25-mm by 0.25- μm film) in splitless mode on a Shimadzu 17A gas chromatograph equipped with a flame ionization detector (FID), an auto sampler AOC-20s, and auto injector AOC-20i. Hydrogen was used as a carrier gas at 1.0 ml/min. Column temperature was held at 120°C for 14 min, programmed to 280°C at $11^{\circ}\text{C}/\text{min}$, and held for 2 min. A sample of Manuka oil was purchased from Coast Biologicals (Auckland South, New Zealand). A sample of Phoebe oil (Weyerstahl et al. 1994) as a source of 7-*epi*-sesquithujene was acquired from the Max Planck

Institute of Chemical Ecology (Jena, Germany). Reference standards of α -cubebene, α -copaene, trans- β -caryophyllene, and α -humulene (α -caryophyllene) were obtained from Sigma-Aldrich Co. (St. Louis, MO).

Electrophysiological Analysis GC-EAD. Two-microliter samples of Manuka oil, Phoebe oil, or an aeration sample of bark tissue from stressed girdled trees (diluted in hexane) were individually injected, splitless, into a HP 6890 GC equipped with a J & W DB-1 column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μ m) equipped with a 1:1 effluent splitter that allowed simultaneous FID and electroantennographic detection (EAD) of the separated volatile compounds. Helium was used as the carrier gas (2.5 ml/min), and the injector temperature was set at 250°C. Oven temperature was held at 45°C for 1 min, programmed to 280°C at 10°C/min, and held for 15 min. The GC outlet for the EAD was set to 220°C to prevent condensation of fractions. The column outlet for the EAD was held in a water-cooled humidified air stream (20°C) flowing at 20 ml/min over an *A. planipennis* antennal preparation. The preparation involved cutting both antennae at the base of the head. The distal half of the tip segment was cut before both antennae were attached to electrodes of an EAG probe (Syntech, Hilversum, the Netherlands), using conductive gel (Spectra 360; Parker Laboratories, Fairfield, NJ). The EAG probe was connected to an IDAC-232 serial data acquisition controller (Syntech). Signals were stored and analyzed on a PC equipped with the program EAD ver. 2.6 (Syntech).

Lure Treatments for Fieldwork. Because of high cost and low availability, alternative sources of some of the EAD-active bark volatiles were needed for fieldwork trials. Manuka oil, a steam distillate from the New Zealand tea tree *Leptospermum scoparium* J.R. and G. Forst (Myrtaceae), known for its antimicrobial activity, contains high amounts of α -cubebene (35 g/liter) α -copaene (48 g/liter), and trans- β -caryophyllene (24 g/liter). Verification of these compounds identification (see Results) and their ratios by GC-MS showed Manuka oil to also contain \approx 3.4 g/liter of α -humulene. Based on these findings and verification that these four compounds in the oil were antennally active by GC-EAD, we decided to use Manuka oil as a lure treatment.

A second natural oil, Phoebe oil, was also tested as a lure treatment. Phoebe oil (Bio-Citrus, Montenegro, Brazil) is a distillate from Brazilian walnut, *Phoebe porosa* Mez., which grows mostly in the Araucaria forests of Parana and Santa Catharina in southern Brazil. It contains the five identified antennally active ash volatiles: the four in Manuka oil plus 7-*epi*-sesquithujene.

Lures were formulated in polypropylene "bubble cap" or in "pouch" devices (Synergy Semiochemicals, Burnaby, British Columbia, Canada). For Manuka oil lures that were designed to release 50 mg total oil/d, release rates of individual compounds were approximately as follows: α -cubebene = 1.6 mg; α -copaene = 2.45 mg; trans- β -caryophyllene = 1.3 mg; α -hu-

mulene = 0.2 mg. For Phoebe oil lures releasing 50 mg total oil/d, individual release rates were approximately as follows: α -cubebene = 0.2 mg; α -copaene = 3 mg; trans- β -caryophyllene = 0.5 mg; α -humulene = 0.8 mg and 7-*epi*-sesquithujene = 0.75 mg. We also tested an ash leaf lure developed by the USDA Forest Service (T. Poland) and the Canadian Forest Service (P. deGroot and G. G. Grant) (Poland et al. 2007) that consisted of four different compounds that were released from individual bubble cap devices at the following daily rates: *cis*-3-hexenol = 3.8 mg, *trans*-2-hexenol = 3.8 mg, *trans*-2-hexenal = 13 mg, and hexanal = 13 mg.

Traps. Three field experiments were conducted in the Michigan counties of Livingston and Ingham from 12 June to 19 July 2007. All trapping experiments used three-panel (35.0 by 58.75 cm each) "prism" traps, constructed of 0.30-cm-thick purple corrugated plastic (described by Francese et al. 2007). Reflectance of purple panels—analyzed with a FieldSpecPro spectrophotometer (Analytical Spectral Devices, Boulder, CO)—exhibited peaks at three wavelengths: 430, 600, and 670 nm. Traps were coated with "brushable" Tangle-trap insect trap coating (The Tanglefoot Company, Grand Rapids, MI). Each lure was hung from a black carabiner (5.63 cm long) and attached to the trap by small holes at the base of the panels.

Manuka Oil Dose Study. We tested traps baited with three release rates against unbaited traps. Nominal release rates (total Manuka oil per day) were 5 (bubble cap), 50 (pouch), and 500 mg (bundle of ten 50 mg pouches). Traps were placed along the edge of ash stands that were infested with *A. planipennis*. We used a complete randomized block design with 12 blocks (trap lines). Traps were suspended from rebar poles (2.4 m long with a 90° bend 45 cm from the top) so that the trap was 1.5 m above the ground. Traps were spaced a minimum of 40 m apart. Trap catch was recorded weekly, and for analysis, numbers of beetles captured on each trap were summed over the entire field season. Lures were rotated within each block weekly, and were replaced after 4 wk. An analysis of variance (ANOVA) was performed on log-transformed data ($n + 1$) with main effects for treatment and block. Tukey's honestly significant difference (HSD) ($\alpha = 0.05$) was used to make pairwise comparisons between treatments.

Comparison of Lures at Two Heights. Nine lines (blocks) of traps were hung at 1.5 and 13 m high along the edges of infested ash stands as described by Francese et al. (2007). Traps were spaced 40 m from each other. Each line had the following four treatments at the two heights: (1) Manuka oil (50 mg/d), (2) a four-component leaf lure, (3) Manuka oil (50 mg/d) plus the four component leaf blend, and (4) an unbaited trap. For each treatment, lures set at 1.5 m always corresponded with the same lure type that was placed 13 m above it.

Lures at both heights were rotated weekly and replaced after 4 wk. Trap catch was recorded weekly, and for analysis, captured beetle counts for each trap height and treatment were summed over the entire

field season. An ANOVA was performed on log-transformed data ($n + 1$) with main effects for lure, height, and block, and an interaction effect between height and lure. Tukey's HSD ($\alpha = 0.05$) was used to compare lures.

Manuka and Phoebe Oil Comparison Study. Twelve trap lines (blocks) were hung along the edge of infested ash tree stands. The lure treatments were (1) 50 mg/d Manuka oil, (2) 50 mg/d Phoebe oil, (3) a combination of both Phoebe oil and Manuka oil (each 25 mg/d), and (4) an unbaited trap.

Traps were suspended from rebar poles at 1.5 m as described above and spaced 40 m apart. Lures were rotated and changed as described above. Caught beetles were counted and summed for each treatment over the entire field season, transformed to log ($n + 1$) and analyzed by ANOVA with main effects for treatment and block. Tukey's HSD ($\alpha = 0.05$) was used to make pairwise comparisons between treatments.

Statistical Analyses. ANOVAs were performed using JMP version 5.1 (SAS Institute 2003) using a general linear model. JMP was also used to perform Tukey's HSD.

Results

GC-MS Analysis. Initial identification of bark volatiles from nongirdled trees showed the presence of monoterpenes, such as α -pinene and limonene, among some nonterpenoid compounds. None of these compounds gave consistent antennal responses in the GC-EAD study. Bark samples taken from the same three trees 24 h after girdling showed a sharp increase in the production of several sesquiterpene hydrocarbons eluting at between 12.00 and 14.00 min (Fig. 1). The most noticeable sesquiterpenes were α -cubebene, α -copaene, and 7-*epi*-sesquithujene (identified by comparison of GC-MS retention times and mass spectra with those of authentic compounds on both DB-1 and HP-5MS columns). Analysis showed that these three compounds exhibited a noticeable increase in production after 24 h. Total bark volatile composition changes were 0–0.6% for α -cubebene, 0.1–3.33% for α -copaene, and 0–0.47% for 7-*epi*-sesquithujene. Two other minor sesquiterpene hydrocarbons were identified by GC-MS in girdled ash bark and showed EAG responses (see below): *trans*- β -caryophyllene and α -humulene.

GC-MS and GC analyses of Manuka oil showed that it consisted of at least 20 compounds. The portion of monoterpenes in the oil was small (total 2.0–2.5%), with α -pinene being the most abundant (0.8%). According to our GC analysis with FID detection, the percentage composition within the Manuka oil of α -cubebene, α -copaene, *trans*- β -caryophyllene, and α -humulene was 4.1, 5.7, 2.6, and 0.4%, respectively. GC analysis of Phoebe oil showed that it had a similar chemical composition to Manuka oil. Phoebe oil contained α -cubebene, α -copaene, *trans*- β -caryophyllene, α -humulene, and 7-*epi*-sesquithujene.

With the exception of α -humulene, the sesquiterpene hydrocarbons identified in girdled ash bark are chiral compounds. α -Cubebene, α -copaene, and *trans*- β -caryophyllene are present in the plant sources in levorotatory forms (Connolly and Hill 1991). To the best of our knowledge, the enantiomeric compositions of these optically active sesquiterpenes in Manuka oil, as well as the chirality of 7-*epi*-sesquithujene in phoebe oil, or any other plant materials, have not been studied. Because we obtained positive antennal responses (see below) from α -cubebene, α -copaene, *trans*- β -caryophyllene, and 7-*epi*-sesquithujene present both in bark aerations and essential oils, we did not pursue their enantiomeric compositions.

Electrophysiological Analysis. In GC-EAD analysis of volatiles from 24 h girdled, green ash tree bark samples, antennae of both male and female *A. planipennis* consistently responded to six compounds in the sesquiterpene range of the GC chromatogram (Fig. 2). Five compounds were identified as α -cubebene, α -copaene, 7-*epi*-sesquithujene, *trans*- β -caryophyllene, and α -humulene by comparison of GC-MS retention times and mass spectra with those of authentic compounds on both DB-1 and DB-5 columns. The sixth compound that gave the final EAD response remains unidentified at present. The approximate ratio of these six EAD active compounds α -cubebene: α -copaene: 7-*epi*-sesquithujene: *trans*- β -caryophyllene: α -humulene: unknown sesquiterpene was 7.1:58.1:27.5: 5.5:1.0:0.8, respectively. Antennal responses ranged from 0.40 to 0.68 mV for females ($n = 5$) and 0.26 to 0.41 mV for males ($n = 3$). 7-*epi*-sesquithujene gave the largest millivolt response for both sexes. Adult beetles showed consistent responses to α -cubebene, α -copaene, *trans*- β -caryophyllene, and α -humulene. A response was measured at 12.1 min, which matched the retention time of our sixth unidentified compound in the ash bark aeration sample. At present, this remains unidentified so is denoted as * (Fig. 2). For Phoebe oil, adult beetles showed the most consistent antennal responses to six compounds, five of which were identified as α -cubebene, α -copaene, 7-*epi*-sesquithujene, *trans*- β -caryophyllene, and α -humulene. The sixth antennal response (12.1 min) once again matched our sixth ash EAD active unidentified compound (Fig. 2).

Manuka Oil Dose Study. Each of the Manuka oil treatments caught significantly more insects than the unbaited treatment ($F = 12.9$; $df = 3,33$; $P < 0.0001$). There was no significant difference in insect catch between the three Manuka oil release rate treatments (Fig. 3).

Comparison of Lures at Two Heights. ANOVA analysis showed that there was no significant interaction between lure treatment and height. Traps at 13 m caught significantly more beetles than traps at 1.5 m ($F = 32.9$; $df = 1,56$; $P < 0.001$). Mean weekly trap catch was 108.8 ± 7.3 on traps at 13 m and 61.8 ± 7.3 on traps at 1.5 m. Manuka oil-baited traps caught more beetles than unbaited control traps at both 1.5 and 13 m (Fig. 4). Traps baited with Manuka oil plus the four-component leaf blend caught significantly more

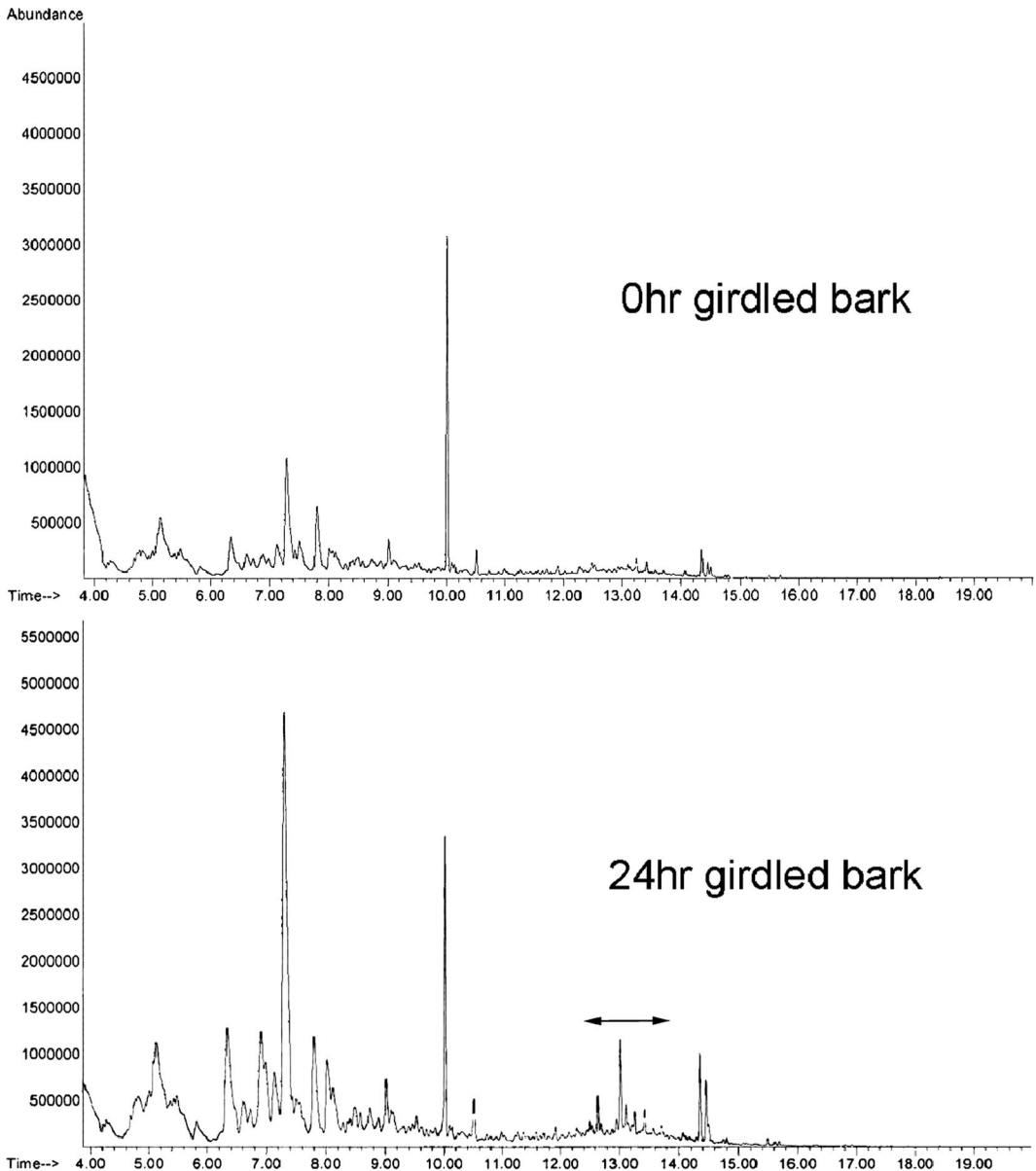


Fig. 1. Chromatograms for emission of volatiles from *F. pennsylvanica* aerations of 0- and 24-h girdled bark. 30 m \times 0.25 mm i.d., 0.25- μ m film DB1 column, at 45°C for 1 min, increasing at 10°C/min to 280°C. Double arrow indicates antennally active sesquiterpene range.

beetles than traps baited with the four-component leaf blend alone or unbaited traps ($F = 10.2$; $df = 3,56$; $P < 0.0001$).

Manuka and Phoebe Oil Comparison Study. Mean capture on traps baited with any of the lure treatments was significantly greater than the catch on unbaited traps ($F = 58.3$; $df = 3,33$; $P < 0.0001$). Mean capture from the Phoebe oil and the Phoebe-Manuka oil combination treatments was significantly higher than that of the Manuka oil or unbaited treatments (Fig. 5). There was no significant difference in beetle catch between traps baited with 50 mg/d Phoebe oil and

traps baited with a combination of 25 mg/d Manuka oil and 25 mg/d Phoebe oil.

Discussion

It is believed that many herbivorous insects have evolved very specific behavioral responses to volatile host-plant chemicals that signal the presence of a host (Miller and Strickler 1984, Visser 1986, Stadler 1992). Our data are the first electrophysiological evidence that male and female *A. planipennis* respond to several

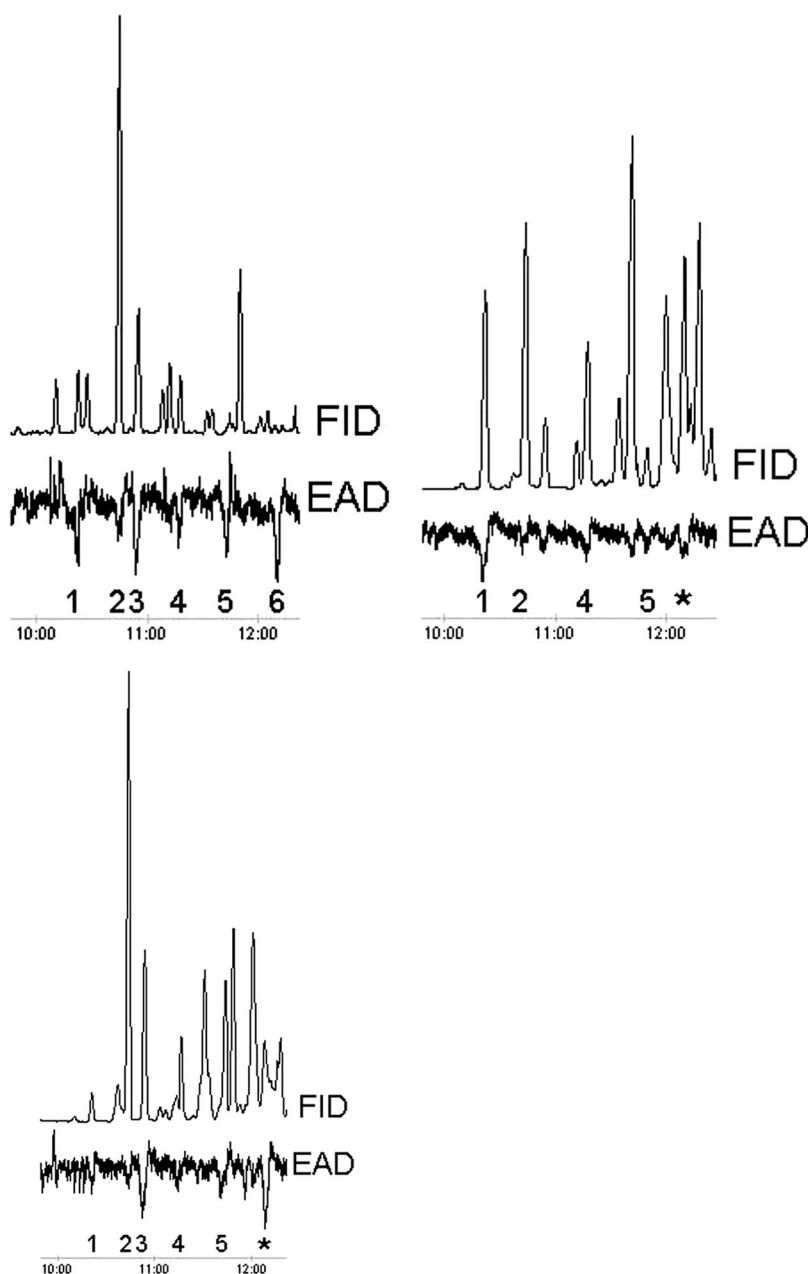


Fig. 2. GC-EAD responses from female *A. planipennis* antenna to compounds emitted from 24-h girdled ash tissue, Manuka oil and Phoebe oil. 30 m \times 0.25 mm i.d., 0.25- μ m film DB1 column, at 45°C for 1 min, increasing at 10°C/min to 280°C ($n = 5$). 1, α -cubebene; 2, α -copaene; 3, 7-*epi*-sesquithujene; 4, *trans*- β -caryophyllene; 5, α -humulene; 6/*, unknown. Retention time shown is between 10 and 12 min.

host bark sesquiterpenes produced by girdled (stressed) green ash. It seems that there is a distinct change in the phloem chemistry of green ash on girdling that causes sudden increases in sesquiterpene compounds that are potentially detected by flying adult *A. planipennis*. These results help explain why girdled trap trees are currently the most efficient tool in trapping *A. planipennis* in surveys and show that adult beetles have the potential to use bark volatiles to help locate stressed

trees. Poland et al. (2005) found that purple cross-vane traps baited with a blend of foliar volatiles that included two of the six GC-EAD active sesquiterpenes, α -caryophyllene (humulene) and *trans*- β -caryophyllene, in addition to pentadecane and *trans*-3-hexenol, captured significantly more beetles than traps baited with individual compounds. These two sesquiterpenes, along with α -cubebene—also found in foliar volatiles from green ash—elicited strong antennal responses (Poland et al.,

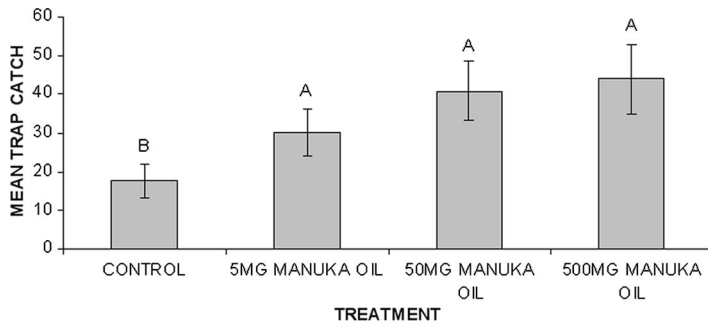


Fig. 3. Mean trap catch (\pm SE) of *A. planipennis* on 1.5-m-high traps baited with lures that provide 5, 50, or 500 mg daily release rates of Manuka oil. Means with different letters indicate significance (Tukey's HSD, $\alpha = 0.05$); analyses performed on data transformed by $\ln(n + 1)$.

unpublished data). Some stress-released sesquiterpenes are therefore systemic, occurring in both ash bark and foliage.

The antennally active bark volatiles identified here have been shown to be potentially important in other plant-insect interactions; for example, α -copaene and β -caryophyllene [along with *p*-cymene, (*E*)- β -ocimene, and γ -terpinene] were shown to be released in large amounts by oak twigs and branches during maturation feeding and formation of maternal galleries by the bark beetle *Scolytus intricatus* (Ratz) (Vrkočová et al. 2000). Attraction to α -cubebene has also been observed for several other beetles in the genus *Scolytus*. The small European elm bark beetle, *S. multistriatus* (Marshall), produces and responds to the pheromones 4-methyl-3-heptanol and (–)- α -multistriatin along with the host-released α -cubebene (Pearce et al. 1975, Lanier et al. 1977, Blight et al. 1980, Millar et al. 1986), whereas the large elm bark beetle, *S. scolytus* (F.) uses only 4-methyl-3-heptanol and α -cubebene (Blight et al. 1978). α -Cubebene, 4-methyl-3-heptanol, and (–)- α -multistriatin have also been reported to attract *S. pygmaeus* (F.) and *S. laevis* (Chapuis) (Minks and Van Deventer 1978, Bejer 1979). The North American native vector of Dutch elm disease, *Hylurgopinus rufipes* (Eichhoff), uses host sesquiterpenes (in particular α -cubebene) to locate moribund

elms in which to breed (Millar et al. 1986). α -Copaene has been shown to be a volatile that emanates from alfalfa seed and has been shown in olfactometer tests to attract the female alfalfa seed chalcid, *Bruchophagus roddi* (Gussakovsky) (Kamm and Buttery 1983). Caryophyllene has been shown to be a major volatile component of cotton and is attractive to a number of insects such as the boll weevil, *Anthonomus grandis* (Boheman) (Minyard et al. 1969, McKibben et al. 1977), the beneficial predatory beetle, *Collops vittatus* (Say) (Flint et al. 1981), and green lacewing, *Chrysopa carnea* (Stephens) (Flint et al. 1979, 1981).

Making a synthetic sesquiterpene single or multi-component lure for *A. planipennis* is not an easy or economically viable option. Sesquiterpenes are difficult and expensive to isolate, identify, and synthesize (Gershenzon 1993). For example, there is much interest in α -copaene as a male lure for Mediterranean fruitfly *Ceratitis capitata* (Wied), because it is two to five times more attractive than the commercially used trimedlure (Cunningham 1989). Unfortunately, current published methods for its synthesis are not economically practical (Heathcock 1966, Heathcock et al. 1967; Corey and Watt 1973). Despite being a fairly common compound throughout the plant kingdom, its natural plant concentration to date has been found to be very low, and no essential oil or plant extract has

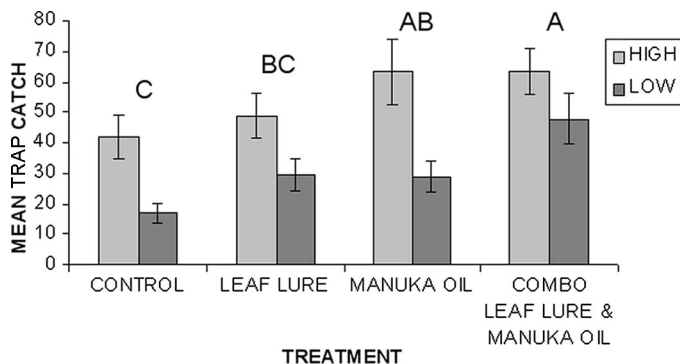


Fig. 4. Mean trap catch (\pm SE) of *A. planipennis* on traps placed at two different heights. Traps were baited with a four-component leaf lure, a 50-mg/d Manuka oil lure, or a combination of the two. Means with different letters indicate significance between lures at both heights (Tukey's HSD, $\alpha = 0.05$); analyses performed on data transformed by $\ln(n + 1)$.

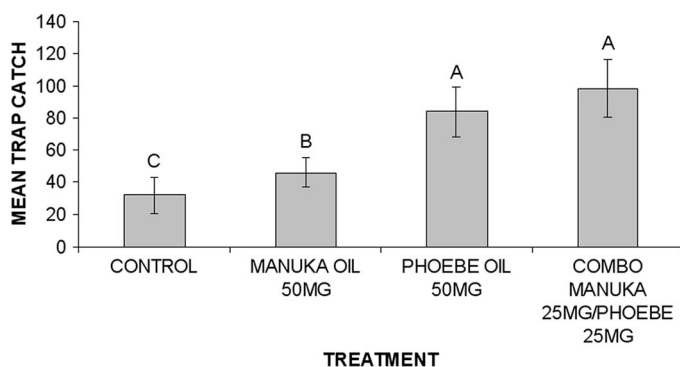


Fig. 5. Mean trap catch (\pm SE) of *A. planipennis* on 1.5-m-high traps baited with 50-mg/d Manuka oil, 50-mg/d Phoebe oil, or a 25-mg Manuka/25-mg/d Phoebe oil combination. Means with different letters indicate significance (Tukey's HSD, $\alpha = 0.05$); analyses performed on data transformed by $1/(n + 1)$.

proven to be a practical source (Flath et al. 1994, Nishida et al. 2000).

Despite containing ~30 other sesquiterpenes and some triketones, namely leptospermone, flavesone, and isoleptospermone, Manuka oil-baited traps consistently caught more beetles than unbaited traps. GC-EAD results for both Manuka and Phoebe oil showed that antennal responses were only consistent within the sesquiterpene retention time range of 10.30–12.10. We are therefore confident that the majority of “non-ash-like” compounds found in these oils do not have negative behavioral effects with regards to trap catch.

Our studies found that adults were attracted to traps baited with oil (ash bark compounds) and leaf lure treatments. Host tree volatiles (especially those released when a tree is stressed) seem to be potentially important host location cues for *A. planipennis*. To date, *A. planipennis* mating success, particularly in the tree canopy, has been shown to rely primarily on visual-, rather than olfactory-, led behavior (Lance et al. 2007, Lelito et al. 2007). Our height study showed that, overall, traps caught more insects when placed at 13 than 1.5 m, indicating that there is more adult insect activity/abundance higher up in an ash canopy. This also supports the previous findings of *A. planipennis* trap placement by Francese et al. (2007). Olfactometer studies have shown that neither sex was attracted to the other, suggesting the lack of a sex or aggregation pheromone (Rodriguez-Saona et al. 2006). Ash volatiles released during periods of tree stress could therefore act as an important means of aggregation for both sexes.

Crude steam distillates from inner bark tissue have been used to catch other *Agrilus* species. Dunn et al. (1986) found that a crude bark distillate of stressed oak trees, when used with sticky-banded trees and vane traps (or with combinations of ethanol and oak volatiles), captured significantly more two-lined chestnut borer, *A. bilineatus* (Weber), than did water controls. Dunn (1985) also showed that *A. bilineatus* were able to locate oaks within 24 h of a stress-inducing injury. Being able to locate stressed ash trees may be beneficial to female *A. planipennis*. Larval *A. planipennis* have been shown to develop faster in heavily

infested trees compared with lightly infested trees (Cappaert et al. 2005).

Our GC-EAD results indicate that Phoebe oil may contain all six compounds that are antennally active in ash bark aerations. In our field test, Phoebe oil caught approximately twice as many beetles as Manuka oil, suggesting that the additional 7-epi-sesquiterpene, along with the unidentified compound (retention time, 12.1 min), may be important cues that adults use to locate stressed ash hosts. Future work will compare attractancy of Phoebe oil combined with leaf-based lures to see if trap catch can be improved even further.

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References Cited

- Bejer, A. B. 1979. Elmebarkbillerne og deres rolle i elmesyngen. Ugeskr. Jordbr. 16–17: 395–398.
- Blight, M. M., C. J. King, L. J. Wadham, and M. J. Wenham. 1978. Attraction of *Scolytus scolytus* (F.) to the components of multilure, the aggregation pheromone of *Scolytus multistriatus* (Marshall) (Coleoptera: Scolytidae). Experimentia 34: 1119–1120.
- Blight, M. M., A. P. Ottridge, L. J. Wadham, M. J. Wenham, and C. J. King. 1980. Response of a European population of *Scolytus multistriatus* to the enantiomers of α -multistriatin. Naturwissenschaften 67: 517–518.
- Cappaert, D., D. G. McCullough, T. M. Poland, and N. W. Siegart. 2005. Emerald ash borer in North America: a

- research and regulatory challenge. *Am. Entomol.* 51: 152–165.
- Carlson, R. W., and F. B. Knight. 1969. Biology, taxonomy, and evolution of four sympatric *Agrilus* beetles (Coleoptera: Buprestidae). *Contrib. Am. Entomol. Inst.* 4: 1–105.
- Connolly, J. D., and R. A. Hill. 1991. Dictionary of terpenoids. Chapman & Hall, London, United Kingdom.
- Corey, E. J., and D. S. Watt. 1973. A total synthesis of (\pm)- α - and (\pm)- β -copaenes and ylangenes. *J. Am. Chem. Soc.* 95: 2303–2311.
- Cote, W. A., and D. C. Allen. 1980. Biology of twolined chestnut borer, *Agrilus bilineatus*, in Pennsylvania and New York. *Ann. Entomol. Soc. Am.* 73: 409–413.
- Cunningham, R. T. 1989. Parapheromones, pp. 221–230. In A. S. Robinson and G. Hooper (eds.), *Fruit flies: their biology, natural enemies and control*, vol. 3A. Elsevier, New York.
- Dunbar, D. M., and G. R. Stephens. 1976. The bionomics of the two lined chestnut borer, pp. 73–86. In J. F. Anderson and H. K. Kaya (eds.), *Perspectives in forest entomology*. Academic, New York.
- Dunn, J. P. 1985. Attraction of the two lined chestnut borer, *Agrilus bilineatus* (Weber), and other associated borers to stressed white oak, *Quercus alba* L. MS thesis, University of Kentucky.
- Dunn, J. P., T. W. Kimmerer, and G. L. Nordin. 1986. Attraction of the twolined chestnut borer, *Agrilus bilineatus* (Weber) (Coleoptera: Buprestidae), and associated borers to volatiles of stressed white oak. *Can. Entomol.* 118: 503–509.
- EAB info. 2007. (<http://www.emeraldashborer.info>.)
- Flath, R. A., R. T. Cunningham, T. R. Mon, and J. O. John. 1994. Male lures for Mediterranean fruitfly (*Ceratitis capitata* Wied.): structural analogs of α -copaene. *J. Chem. Ecol.* 20: 2595–2609.
- Flint, H. M., S. S. Salter, and S. Walters. 1979. Caryophyllene: an attractant for the green lace wing. *Environ. Entomol.* 8: 1123–1125.
- Flint, H. M., J. R. Merkle, and M. Sledge. 1981. Attraction of male *Collops vittatus* in the field by caryophyllene alcohol. *Environ. Entomol.* 10: 301–304.
- Francese, J. A., V. C. Mastro, J. B. Oliver, D. R. Lance, N. Youssef, and S. G. Lavalley. 2005. Evaluation of colors for trapping *Agrilus planipennis* (Coleoptera: Buprestidae). *J. Entomol. Sci.* 40: 93–95.
- Francese, J. A., I. Fraser, D. R. Lance, and V. C. Mastro. 2007. Developing survey techniques for emerald ash borer: the role of trap height and design, pp. 72–73. In V. C. Mastro, D. Lance, R. Reardon, and G. Parra (eds.), *Emerald ash borer research and technology development meeting*. US Department of Agriculture Forest Health Technology Enterprise Team, October 29–November 2 2006, Cincinnati, OH.
- Gershenson, J. 1993. The cost of plant chemical defenses against herbivory: a biochemical perspective, pp. 105–173. In E. A. Bernays (ed.), *Insect-plant interactions*, Vol. V. CRC, Boca Raton, FL.
- Gwynne, D. T., and D.C.F. Rentz. 1983. Beetles on the bottle: male buprestids mistake stubbies for females (Coleoptera). *J. Aust. Entomol. Soc.* 23: 79–80.
- Haack, R. A., and D. M. Benjamin. 1982. The biology and ecology of the twolined chestnut borer, *Agrilus bilineatus*, on oaks, *Quercus* spp in Wisconsin. *Can. Entomol.* 114: 385–395.
- Haack, R. A., E. Jendek, H. Liu, K. R. Marchant, T. R. Petrice, T. M. Poland, and H. Ye. 2002. The Emerald ash borer: a new exotic pest in North America. *Newslett. Mich. Entomol. Soc.* 47: 1–5.
- Heathcock, C. H. 1966. The total synthesis of (\pm)-copaene and (\pm)-8-isocopaene. *J. Am. Chem. Soc.* 88: 4110–4112.
- Heathcock, C. H., R. A. Badger, and J. W. Patterson, Jr. 1967. Total synthesis of (\pm)-copaene and (\pm)-ylangene. A general method for the synthesis of tricyclic [4.4.0.0^{2,7}] decanes. *J. Am. Chem. Soc.* 89: 4133–4145.
- Kamm, J. A., and R. G. Buttery. 1983. Response of the alfalfa seed chalcid *Bruchophagus roddi*, to alfalfa volatiles. *Entomol. Exp. Appl.* 33: 129–134.
- Lance, D. R., I. Fraser, and V. C. Mastro. 2007. Activity and microhabitat-selection patterns for emerald ash borer and their implications for the development of trapping systems, pp. 77–78. In V. C. Mastro, D. Lance, R. Reardon, and G. Parra (eds.), *Emerald ash borer research and technology development meeting*. US Department of Agriculture Forest Health Technology Enterprise Team, October 29–November 2 2006, Cincinnati, OH.
- Lanier, G. N., W. E. Gore, G. T. Pearce, J. W. Peacock, and R. M. Silverstein. 1977. Response of the European elm bark beetle, *Scolytus multistriatus* (Coleoptera: Scolytidae), to isomers and components of its pheromone. *J. Chem. Ecol.* 13: 1045–1067.
- Lelito, J. P., J. H. Tumlinson, and T. C. Baker. 2007. Visually mediated paratrooper copulations in the mating behavior of *Agrilus planipennis* (Coleoptera: Buprestidae), pp. 9. In V. C. Mastro, D. Lance, R. Reardon, and G. Parra (eds.), *Emerald ash borer research and technology development meeting*. US Department of Agriculture Forest Health Technology Enterprise Team, October 29 to November 2, 2006, Cincinnati, OH.
- Liu, H., L. S. Bauer, R. Gao, T. Zhao, T. R. Petrice, and R. A. Haack. 2003. Exploratory survey for the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), and its natural enemies in China. *Great Lakes Entomol.* 36: 191–204.
- Marchant, K. R. 2004. Emerald ash borer in Canada–2003, pp. 3. In V. C. Mastro and R. Reardon (eds.), *Emerald ash borer research and technology development meeting*. US Department of Agriculture Forest Health Technology Enterprise Team, September 30 to October 1 2003, Port Huron, MI.
- Marchant, K. R. 2006. Managing the emerald ash borer in Canada–2005, pp. 3. In V. C. Mastro, R. Reardon, and G. Parra (eds.), *Emerald ash borer research and technology development meeting*. US Department of Agriculture Forest Health Technology Enterprise Team, September 26–27 2005, Pittsburgh, PA.
- McKibben, G. H., E. Mitchell, W. P. Scott, and P. A. Hedin. 1977. Boll weevils are attracted to volatile oils from cotton plants. *Environ. Entomol.* 6: 804–806.
- Millar, J. G., C. H. Zhao, G. N. Lanier, D. P. O'Callaghan, M. Griggs, J. R. West, and R. M. Silverstein. 1986. Components of moribund American elm trees as attractants to elm bark beetles, *Hylurgopinus rufipes* and *Scolytus multistriatus*. *J. Chem. Ecol.* 12:583–608.
- Miller, J. R., and K. L. Strickler. 1984. Finding and accepting host plants, pp. 127–157. In W. J. Bell, and R. T. Cardé (eds.), *Chemical ecology of insects*. Chapman & Hall, New York.
- Minks, A. K., and P. Van Deventer. 1978. Fenologische waarnemingen van iepespintkevers gedurende 1975 en 1976 in Nederland met behulp van attractantia. *Ned. Bosbouw Tijdschr.* 50:151–158.
- Minyard, J. P., D. D. Hardee, R. C. Gueldner, A. C. Thompson, G. Wiygul, and P. A. Hedin. 1969. Constituents of

- the cotton flower bud. Compounds attractive to the boll weevil. *J. Agric. Food Chem.* 17: 1093–1097.
- Nishida, R., T. E. Shelly, T. S. Whittier, and K. Y. Kaneshiro. 2000. α -copaene, a potential rendezvous cue for the mediterranean fruit fly, *Ceratitis capitata*? *J. Chem. Ecol.* 26: 87–100.
- Oliver, J. B., Y. Youseef, D. Fare, M. Halcomb, S. Scholl, W. Klingeman, and P. Flanagan. 2002. Monitoring buprestid borers in production nursery areas, pp. 17–23. *In* G. Haun (ed.), *Proceedings of the 29th Annual Meeting of the Tennessee Entomological Society*.
- Pearce, G. T., W. E. Gore, R. M. Silverstein, J. W. Peacock, R. A. Cuthbert, G. N. Lanier, and J. B. Simeone. 1975. Chemical attractants for the smaller European elm bark beetle, *Scolytus multistriatus*. *J. Chem. Ecol.* 1: 115–124.
- Poland, T. M., D. S. Purewaran, G. Grant, and P. de Groot. 2007. Field attraction of emerald ash borer to antennally and behaviorally active ash volatiles, pp. 80–81. *In* V. C. Mastro, D. Lance, R. Reardon, and G. Parra (eds.), *Emerald ash borer research and technology development meeting*. U.S. Department of Agriculture Forest Health Technology Enterprise Team, 29 October–2 November 2006, Cincinnati, OH.
- Poland, T. M., and D. G. McCullough. 2006. Emerald ash borer: Invasion of the urban forest and the threat to North America's ash resource. *J. For.* 104: 118–124.
- Poland, T. M., P. de Groot, G. G. Grant, L. MacDonald, and D. G. McCullough. 2004. Developing attractants and trapping techniques for the emerald ash borer, pp. 15–16. *In* V. C. Mastro and R. Reardon (eds.), *Emerald ash borer research and technology development meeting*. US Department of Agriculture Forest Health Technology Enterprise Team, September 30 to October 1 2003, Port Huron, MI.
- Poland, T. M., D. G. McCullough, P. de Groot, G. G. Grant, L. MacDonald, and D. L. Cappaert. 2005. Progress toward developing trapping techniques for the emerald ash borer, pp. 53–54. *In* V. C. Mastro and R. Reardon (eds.), *Emerald ash borer research and technology development meeting*. US Department of Agriculture Forest Health Technology Enterprise Team, October 5–6 2004, Romulus, MI.
- Rauscher, K., and V. C. Mastro. 2004. The Michigan emerald ash borer program, pp. 1–2. *In* V. C. Mastro and R. Reardon (eds.), *Emerald ash borer research and technology development meeting*. US Department of Agriculture Forest Health Technology Enterprise Team, September 30 to October 1, 2003, Port Huron, MI.
- Rodriguez-Saona, C., T. M. Poland, J. R. Miller, L. L. Stelinski, G. G. Grant, P. De Groot, L. Buchan, and L. MacDonald. 2006. Behavioral and electrophysiological responses of the emerald ash borer, *Agrilus planipennis*, to induced volatiles of Manchurian ash, *Fraxinus mandshurica*. *Chemoecology* 16:75–86.
- SAS Institute. 2003. JMP version 5.1. SAS Institute, Cary, NC.
- Stadler, B. 1992. Physiological responses of *Uroleucon jaceae* (L.) to seasonal changes in the quality of its host plant *Centaurea jacea* L.: multilevel control of adaptations to the life cycle of the host. *Oecologia (Berl.)* 91: 273–280.
- U.S. Department of Agriculture Natural Resources Conservation Service [USDA-NRCS]. 2004. The PLANTS database, version 3.5. National Plant Data Center, Baton Rouge, LA.
- Visser, J. H. 1986. Host odor perception in phytophagous insects. *Annu. Rev. Entomol.* 31: 121–144.
- Vrkočová, P., I. Valterova, J. Vrkoč, and B. Koutek. 2000. Volatiles released from oak, a host tree for the bark beetle *Scolytus intricatus*. *Biochem. Syst. Ecol.* 28:933–947.
- Wei, X., D. Reardon, Y. Wu, and J. H. Sun. 2004. Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in China: a review and distribution survey. *Acta Entomol. Sin.* 47: 679–685.
- Weyerstahl, P., H. C. Wahlburg, U. Splittgerber, and H. Marschall. 1994. Volatile constituents of brazilian phoebe oil. *Flavour Frag. J.* 9: 179–186.
- Yu, C. M. 1992. *Agrilus marcopoli* Obenberger, pp. 400–401. *In* G.-R. Xiao (ed.), *Forest insects of China*, 2nd ed. China Forestry Publishing House, Beijing, China.
- Zhang, Q. E., F. Schlyter, and G. Birgersson. 2000. Bark volatiles from nonhost angiosperm trees of spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytidae): chemical and electrophysiological analysis. *Chemoecology* 10: 69–80.

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